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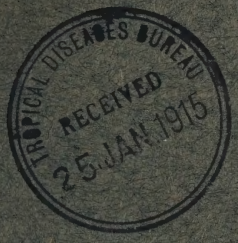
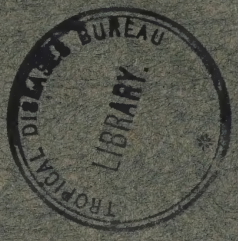
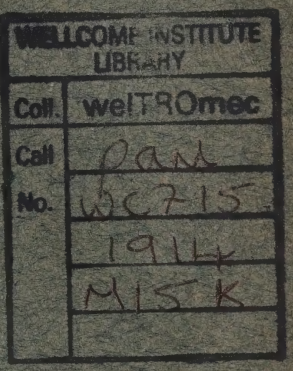
KALA-AZAR IN NOWGONG (ASSAM)





BY

CAPTAIN F. PERCIVAL MACKIE, M.B., F.R.C.S., M.R.C.P., I.M.S



FROM

THE INDIAN JOURNAL OF MEDICAL RESEARCH, VOL. I, NO. 4, APRIL, 1914

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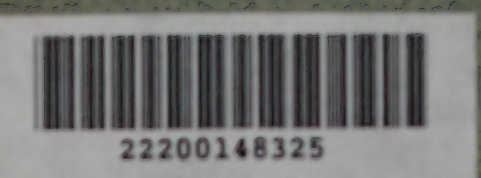
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KALA-AZAR IN NOWGONG (ASSAM).

BY

CAPTAIN F. PERCIVAL MACKIE, M.B., F.R.C.S., M.R.C.P., I.M.S.

(Received for publication, 25th January 1914.)*

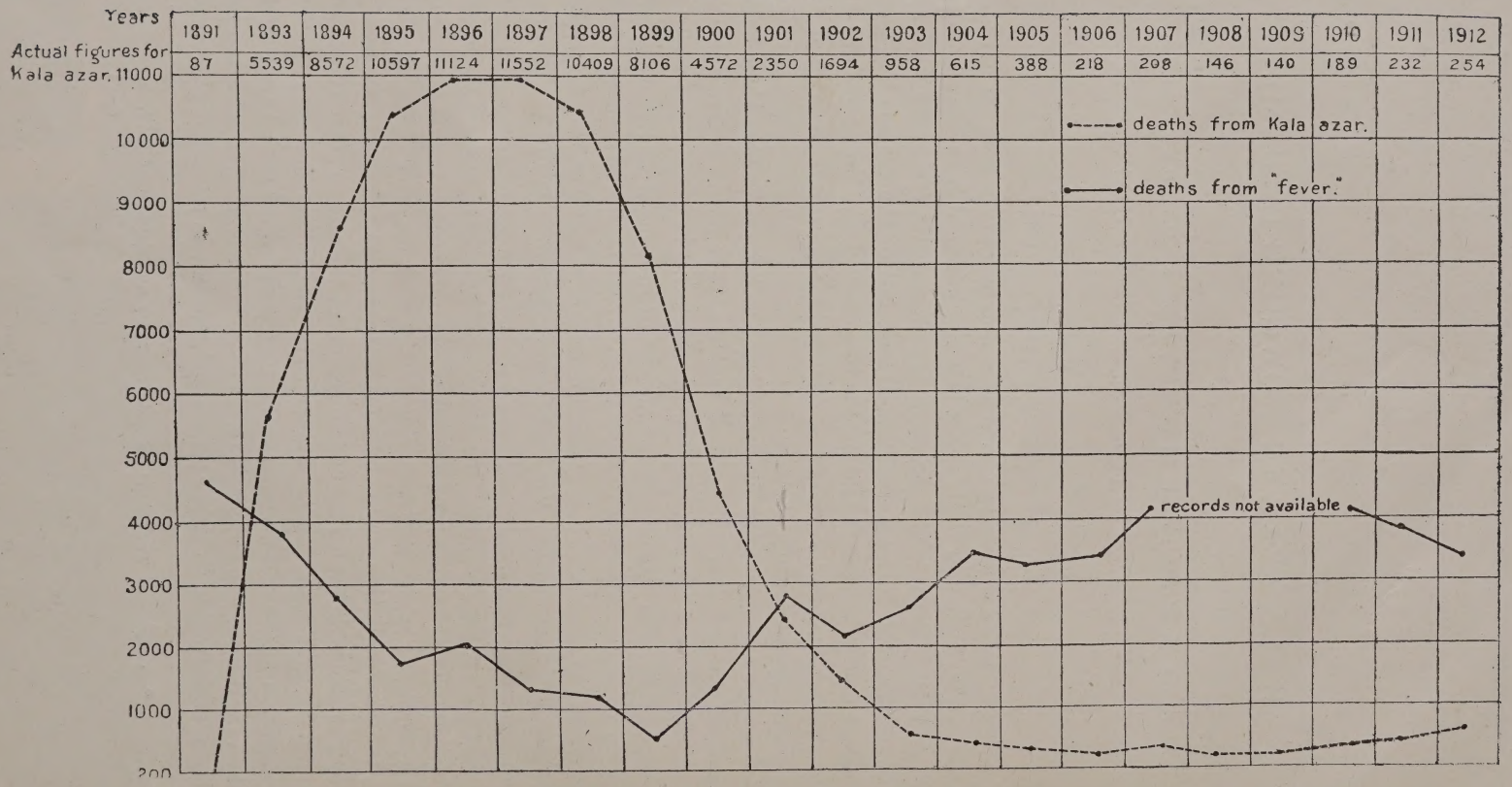
EPIDEMIOLOGICAL OBSERVATIONS.

THE disease is stated to have appeared in Nawkhola, in the extreme west of the district, in 1891, where it was introduced from the adjoining district of Goalpara, and to have spread rapidly eastwards through the district of Nowgong. The rise and fall of the disease has been studied month by month and *mauza* by *mauza* from the year 1891 to the present time and some of the principal features have been recorded in curves in the attached tables.

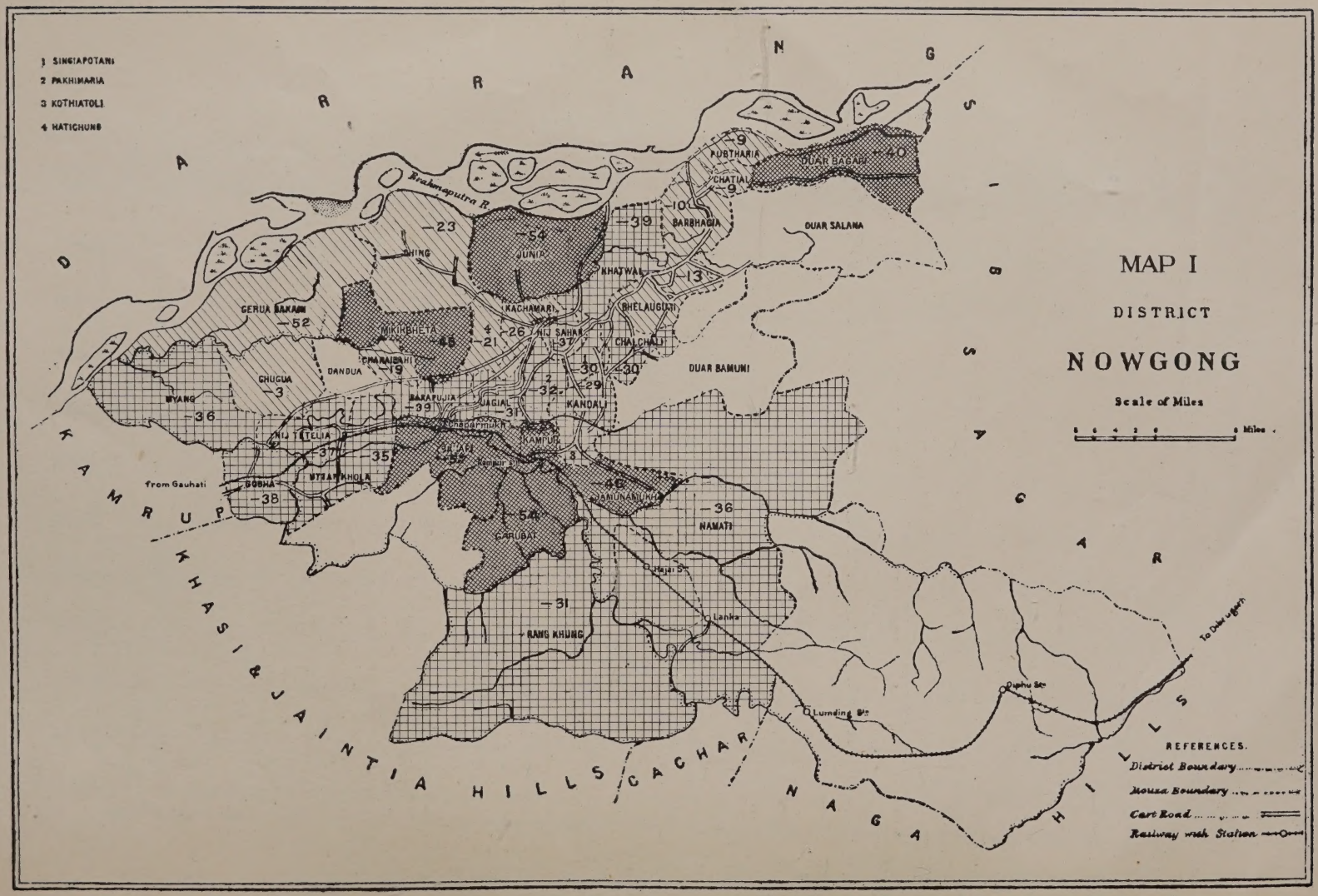
At the same time the figures relating to "fever" other than kala-azar, which is taken to represent malaria, have been examined in the same detail over the same period, to ascertain whether the two diseases bear any relation to one another in time or in general distribution. A short explanatory description is attached to each table and map, to which attention is called. It is important to know whether the mortality returns are now shewing any considerable recrudescence of the disease, but the curves and figures in Chart I do not bear out the idea that the disease is on the increase. At the same time there is a very general belief amongst the villagers that the disease is becoming more frequent and when one remembers how chronic the sporadic type of the disease is, it is probable that any considerable increase in the mortality would not be noticeable till a year or two following the rise in infectivity had begun to take effect. Map I shews the percentage decrease in the population in the various *mauzas* during the decennial period 1891-1901 when the disease was at its height. The decrease is allowed by all to be almost entirely due to kala-azar, as emigration was not large, and there

* A report of work done between February and September 1912.

CHART I.



Showing the actual number of deaths from kala-azar and from " fever " in the District of Nowgong, year by year, from 1891—1912.



Represents the decrease in population in the various mauzas reckoned in percentages of the total village population between the years 1891—1901 when the epidemic of Kala-azar was at its height.

Heavy shaded = A reduction in population of 40% or over. Medium shaded = Reduction of between 30%—40%.
Light shaded = ,, ,, ,, under 36%. Blank = No information. Actual figures are written in each mauza.

was no epidemic other than kala-azar to account for it. The Settlement Officers who worked in the *mofussil* during the height of and after the epidemic speak eloquently of the widespread mortality and desolation caused by its ravages. The decrease in population varied from 3·46% in the *mauza* Ghagua to 55% in the *mauzas* of Kothiatoli, Kampur and Juria. The conditions in the various areas have been examined, to ascertain, if possible, why the blow fell so much more heavily on one than on another, but at this length of time it is untrustworthy to draw any deductions, where so many factors would have to be considered. Map II shews the recovery in the population per cent. in the decade 1901-1911 following the epidemic. As far as one can ascertain by village inspection, there is a very large juvenile population which has sprung up in the last ten or twelve years, a condition which results in the increase of inflammable material.

Maps III and IV shew the present distribution of kala-azar in the district according to the return of the *mauzadars* and those verified by myself and my staff.*

These maps have only a limited value, for it has not been found possible to get figures which would represent the deaths per cent. of population.

The Survey of the Nowgong Municipality.—A kala-azar census of the whole township was made and the following details asked for in each house:—

I.—Number or identification mark of house.
II.—Present occupier.
III.—Religion or caste.
IV.—Race.
V.—Occupation.
VI.—How long resident in Nowgong and from whence come.
VII.—Number of persons in house over 15 years of age.
Ditto ditto under 15 years of age.
VIII.—General dietary—
(*a*) Mixed.
(*b*) Purely vegetarian.

---

* Note that in these maps each disc represents one living case of kala-azar, whereas in Map V each disc represents 50 deaths from kala-azar in the year, and in Map VI each disc represents 10 deaths from "fever" in the year referred to.

IX.—Water-supply for drinking, and for domestic purposes—

(*a*) Open river Kallang.

(*b*) Open tank.

(*c*) Well or pump.

X.—Whether persons of household go to river Kallang to bathe or to wash clothes.

XI.—*Kala-azar.*—Number of cases in house with details. Deaths in house during the last three years from kala-azar. If kala-azar is stated to be present, give the duration of the disease and clinical diagnosis, whether "probable" or "doubtful."

XII.—Insects present in house.

Under this scheme the population investigated numbered 4,778 persons, of which 27 suffered from "positive" kala-azar, 21 were "suspicious," and 64 deaths from kala-azar were reported to have occurred during the last three years.

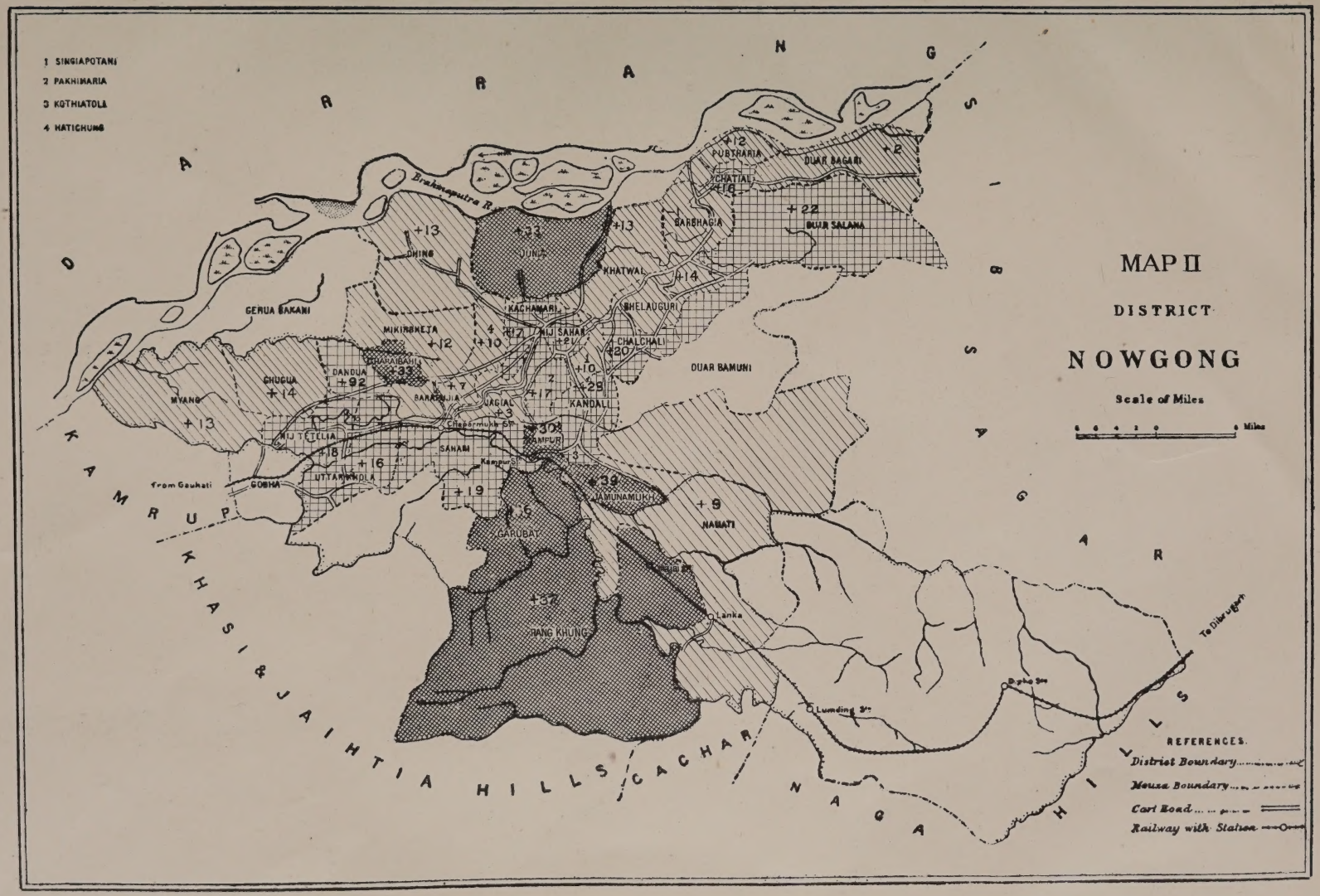
The number of cases is thus very small and is not large enough to base any statistical conclusions on the various questions enquired into, but it is hoped that the work will be useful in deciding whether there is any considerable increase in the disease in the next few years.

One interesting point was reported, namely, that amongst the Jain community, which numbers about 200, there is no case of kala-azar, and they affirm positively that no case of the disease has ever occurred amongst them, not even during the height of the past epidemic. They attribute their immunity to their adherence to a vegetarian diet and particularly to their avoidance of fish. I made many enquiries to test the truth of this statement, and though people were found who doubted their immunity on the one hand, and their strict vegetarian principles on the other, I have not been able to get any evidence of the death of a Jain from kala-azar.

The township of Nowgong suffered considerably from kala-azar in the 1891-1901 decade as the following table shews :—

Census.	Total population.	Variation.
1872	3,241	....
1881	4,248	+ 1,007
1891	4,815	+ 567
1901	4,430	− 385
1911	5,433	+ 1,003

The survey of the municipality was entrusted to Sub-Assistant Surgeon Didaruddin Ahmed, who was lent to me by the Assam Govern-



Represents the percentage recovery of the population mauza by mauza during the decennial period 1901—1911 following the epidemic of Kala-azar in the previous decade.
Heavy shaded=Over 30% recovery. Medium shaded=From 15%—30% recovery. Light shaded= Under 15% recovery Blank=No information.
The actual percentages of increase of population are inserted in the respective mauzas.

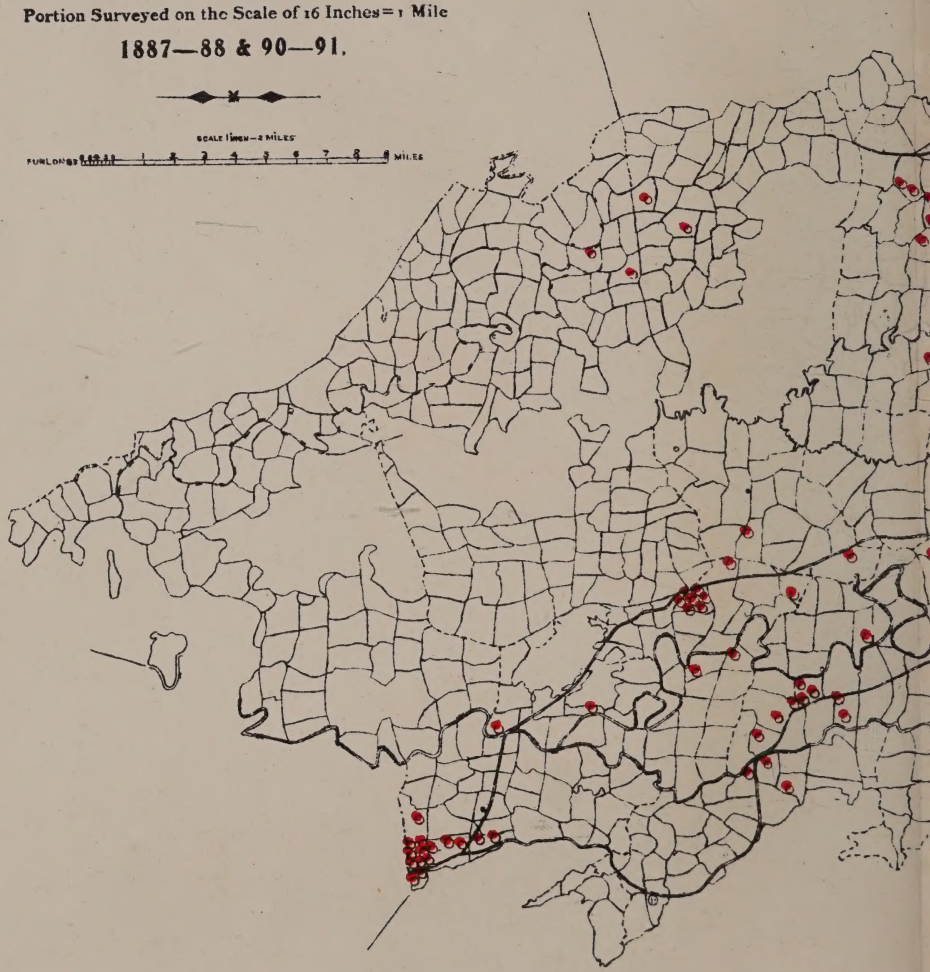
MAP III

District

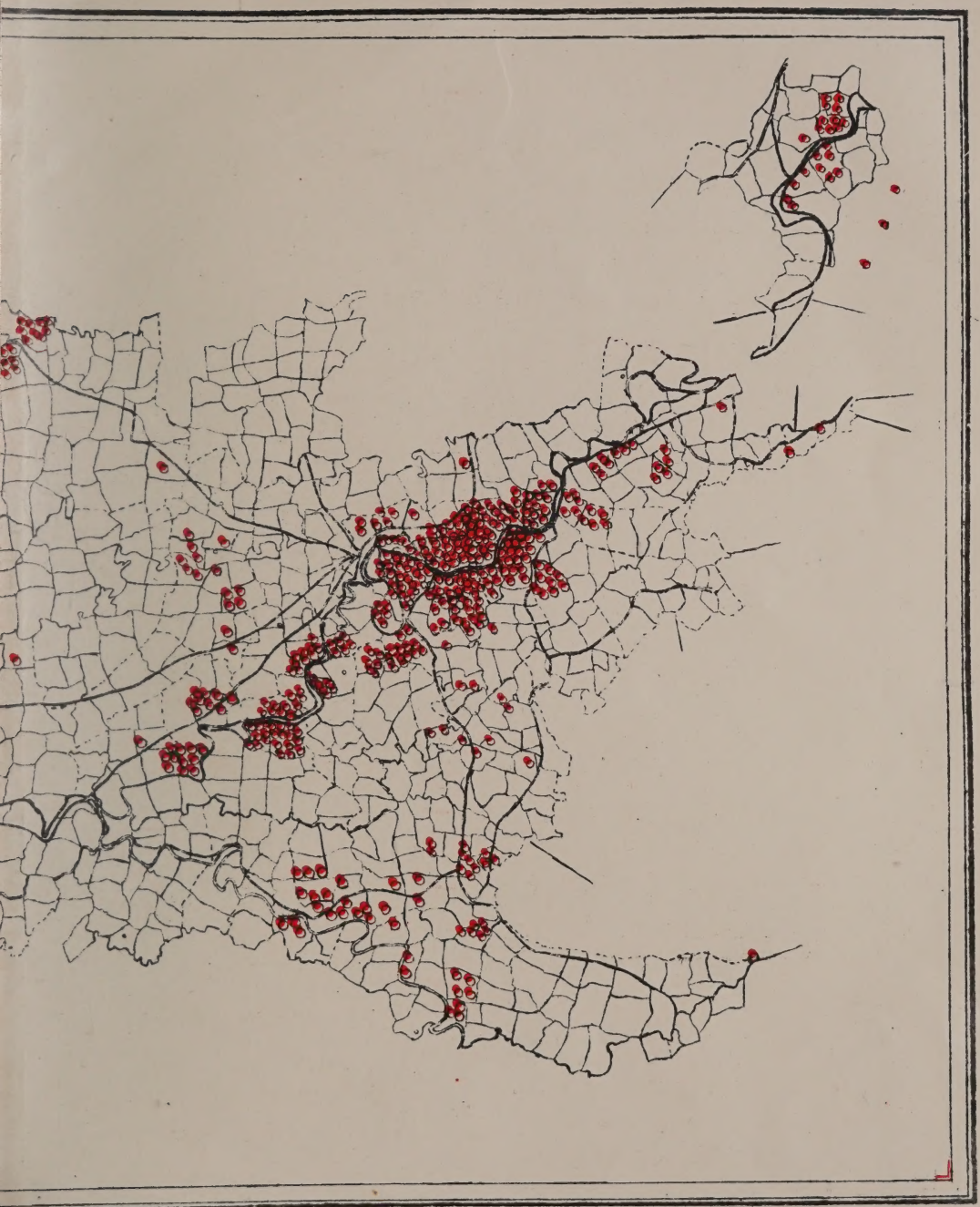
NOWGONG

Portion Surveyed on the Scale of 16 Inches = 1 Mile

1887—88 & 90—91.



Distribution of Kala-Azar
returns of mauzadars
Each red disc refers to a
Blank villages returned as



Feb. 1913, according to the village officials.

of Kala-Azar.

infected

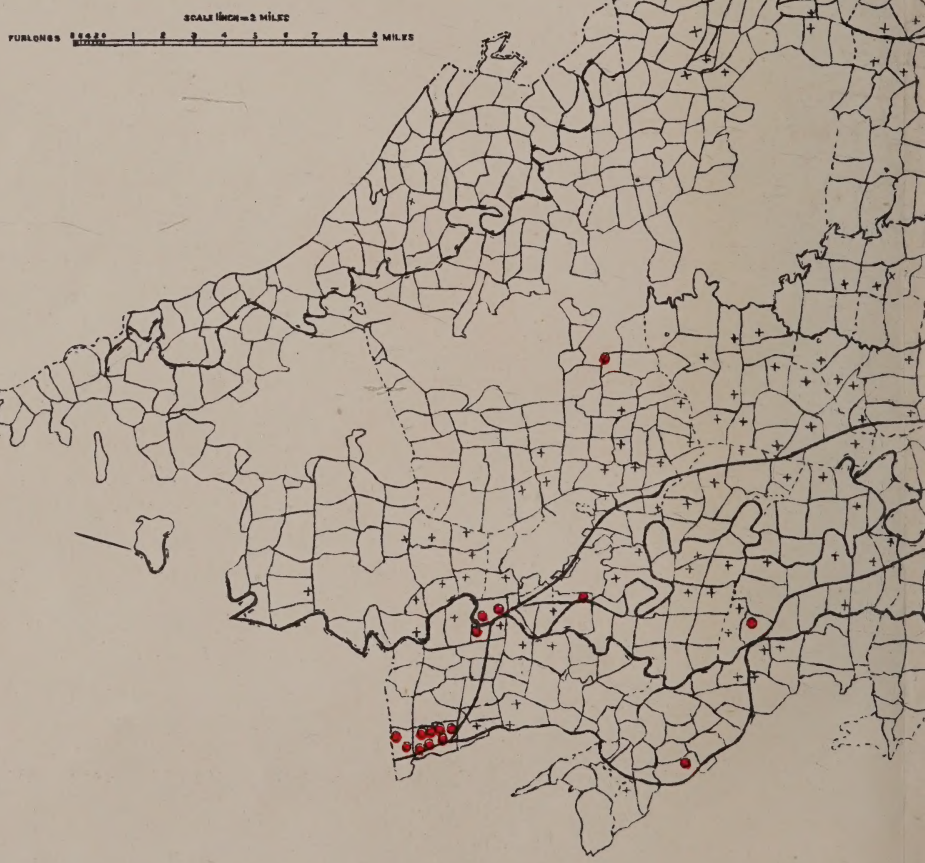
MAP IV

District

NOWGONG

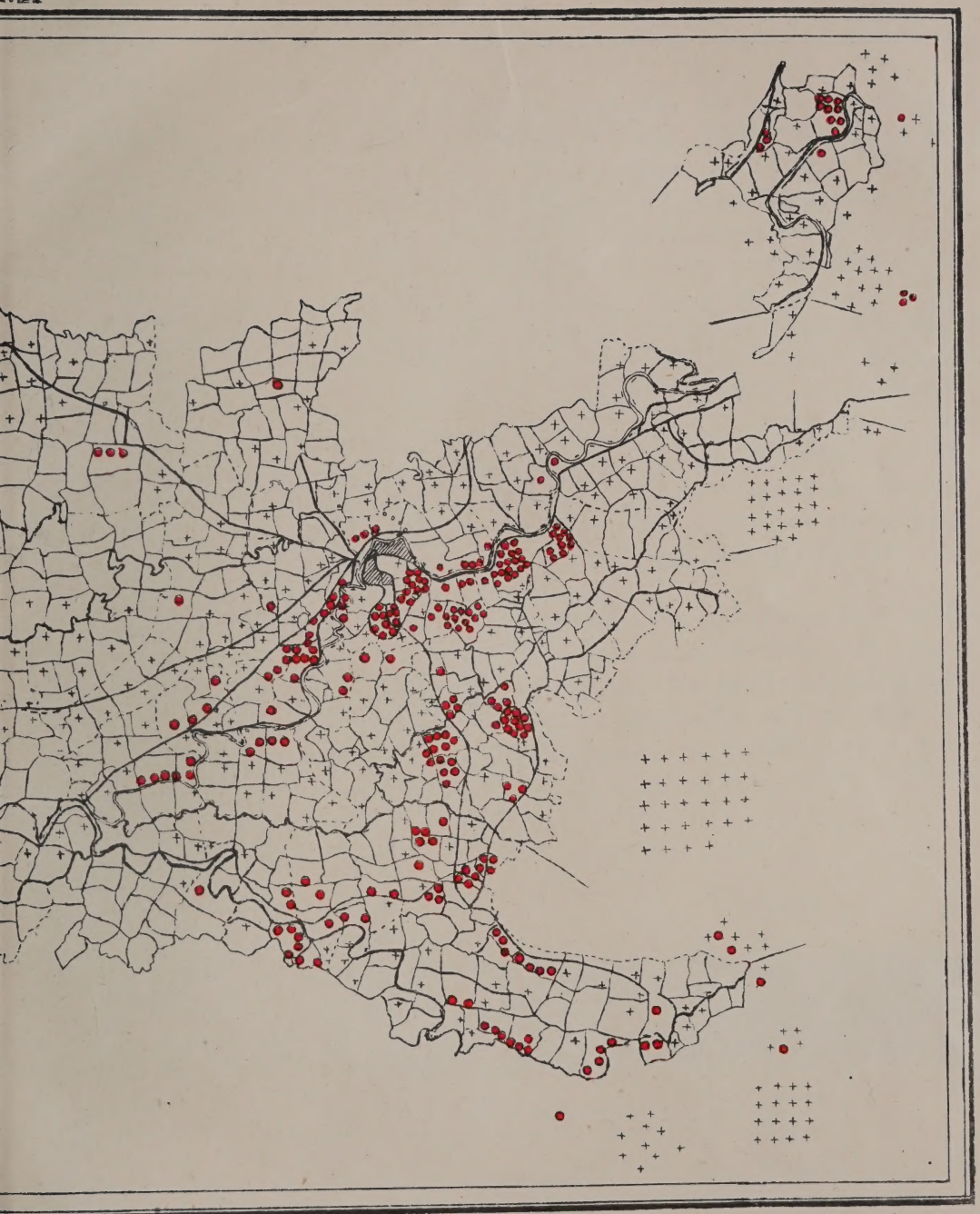
Portion Surveyed on the Scale of 16 Inches = 1 Mile

1887—88 & 90—91.



The Red discs represent

The black crosses represe
reported as being fre



of Kala Azar.

llages inspected and
n Kala Azar.

ment, and I am indebted to him for tactfully carrying out this tedious piece of work.

Detailed surveys have also been carried out by me and my staff in the Kachari group of villages in Kamrup and in the Golaghat subdivision. A detailed one is being prepared for the highly infected village of Nartamgaon near Nowgong, and I propose doing the same for some infected foci in other parts of Assam during the ensuing season.

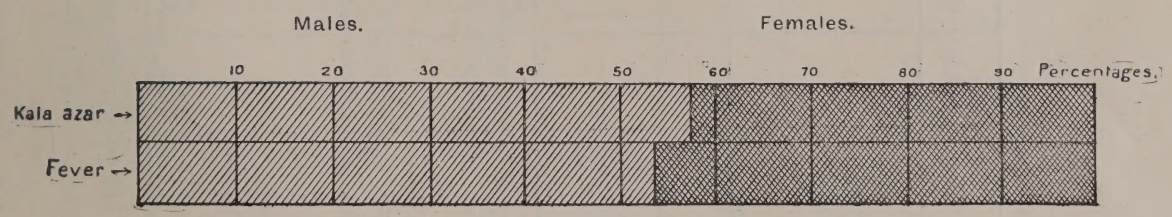
Seasonal Prevalence.—I have not been able to find reliable evidence as to any special seasonal prevalence. This is partly due to the difficulty of fixing the onset of a disease of unknown incubation period especially when the onset itself is so insidious. The distribution of deaths according to months has been plotted out for the period of 19 years, both for kala-azar and for "fever" (see Chart II), but this does not help in fixing the seasonal prevalence of the invasion period.

Incidence according to occupation.—No special liability of occupation could be proved. This is partly due to the fact that the vast majority of Assamese are agriculturists, and even when they do other work, they prefer to describe themselves as such.

Incidence according to age and sex.—The ages are not given in the mortality returns, so I have only figures from 195 of my own series of verified cases. Chart III represents the age distribution among these cases in graphic form. This shews that roughly half the cases are between 5—10 years of age and 81·5% in the first fifteen years of life.

Chart IV shews the distribution according to sex and indicates that the sexes are about equally liable.

CHART IV.

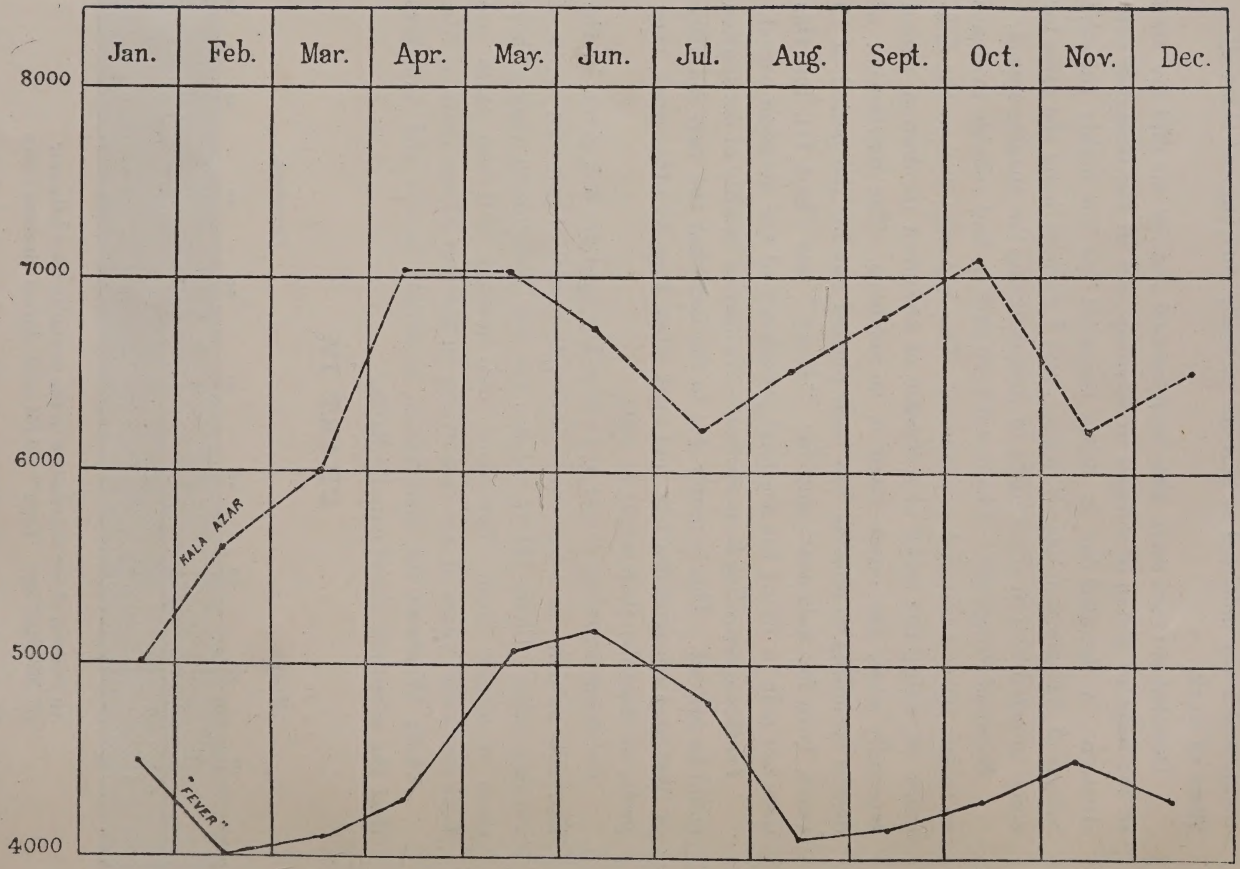


An analysis of *sex distribution* in the mortality from kala-azar (77,962 total) and "fever" (56,784 total) during nineteen years.

The Meteorological Conditions of Nowgong.—Chart V shews the average annual rainfall by months, calculated for five recent years.

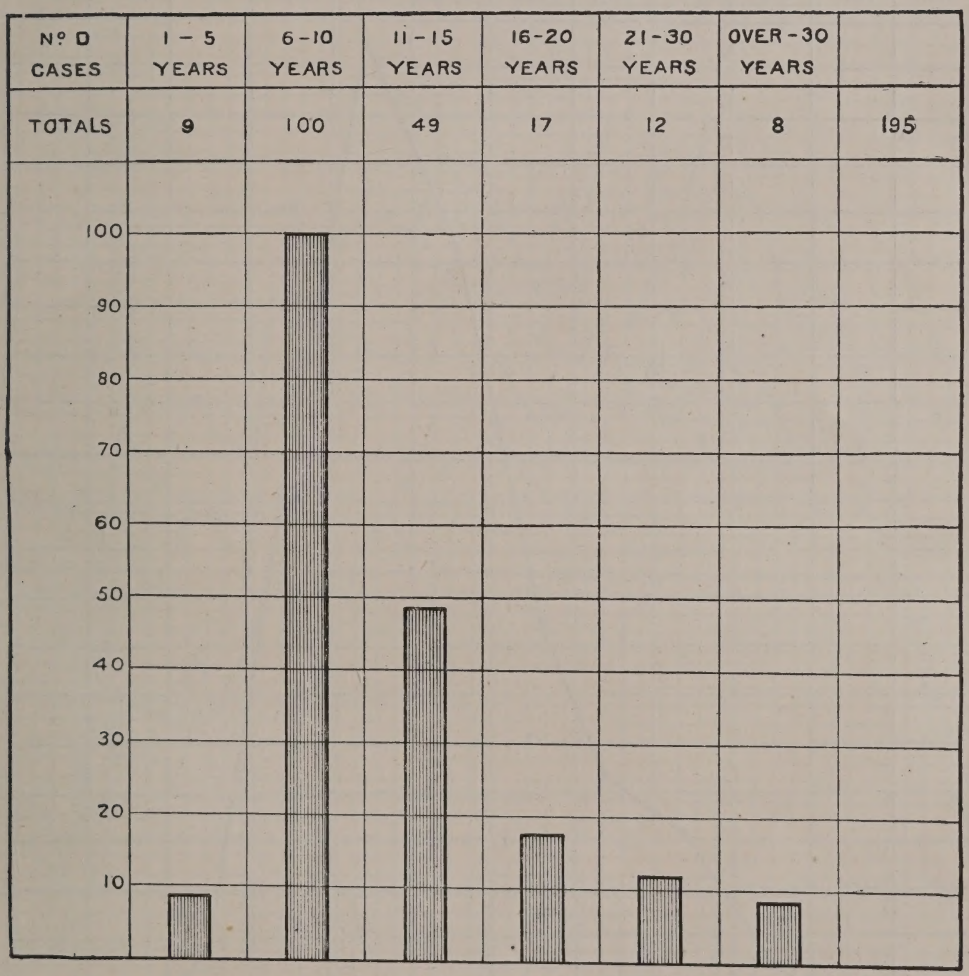
Chart VI represents the maximum and minimum dry bulb and wet bulb temperature records for a year. It has been

CHART II.



Shewing the total mortality during nineteen years from kala-azar (dotted) and "fever" (plain) according to monthly distribution.

CHART III.



Shewing the age distribution in 195 cases of kala-azar.

CHART V.

Rainfall in Nowgong Town taken from the average of five normal years.
Average annual fall from 70—80 inches.

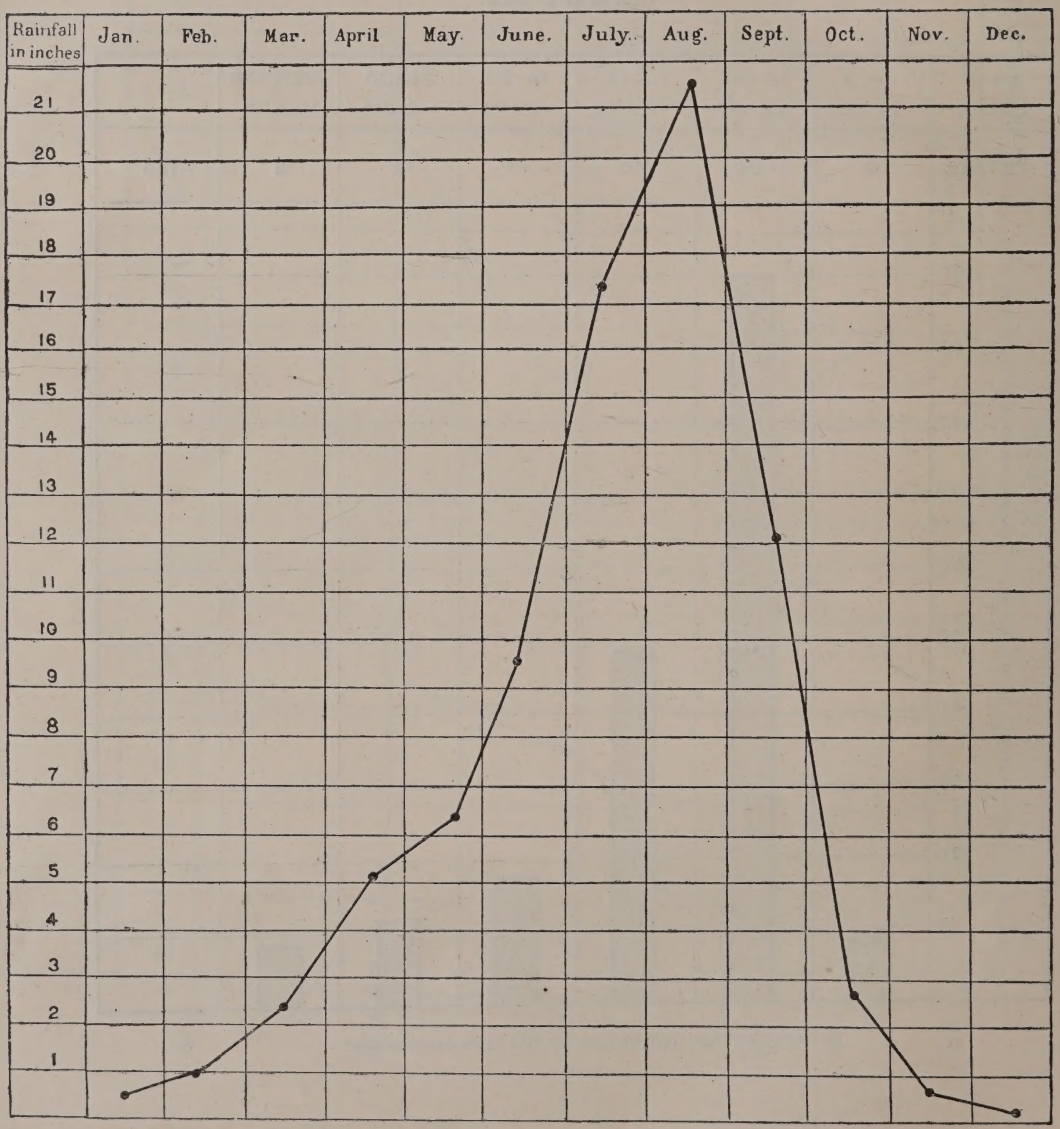
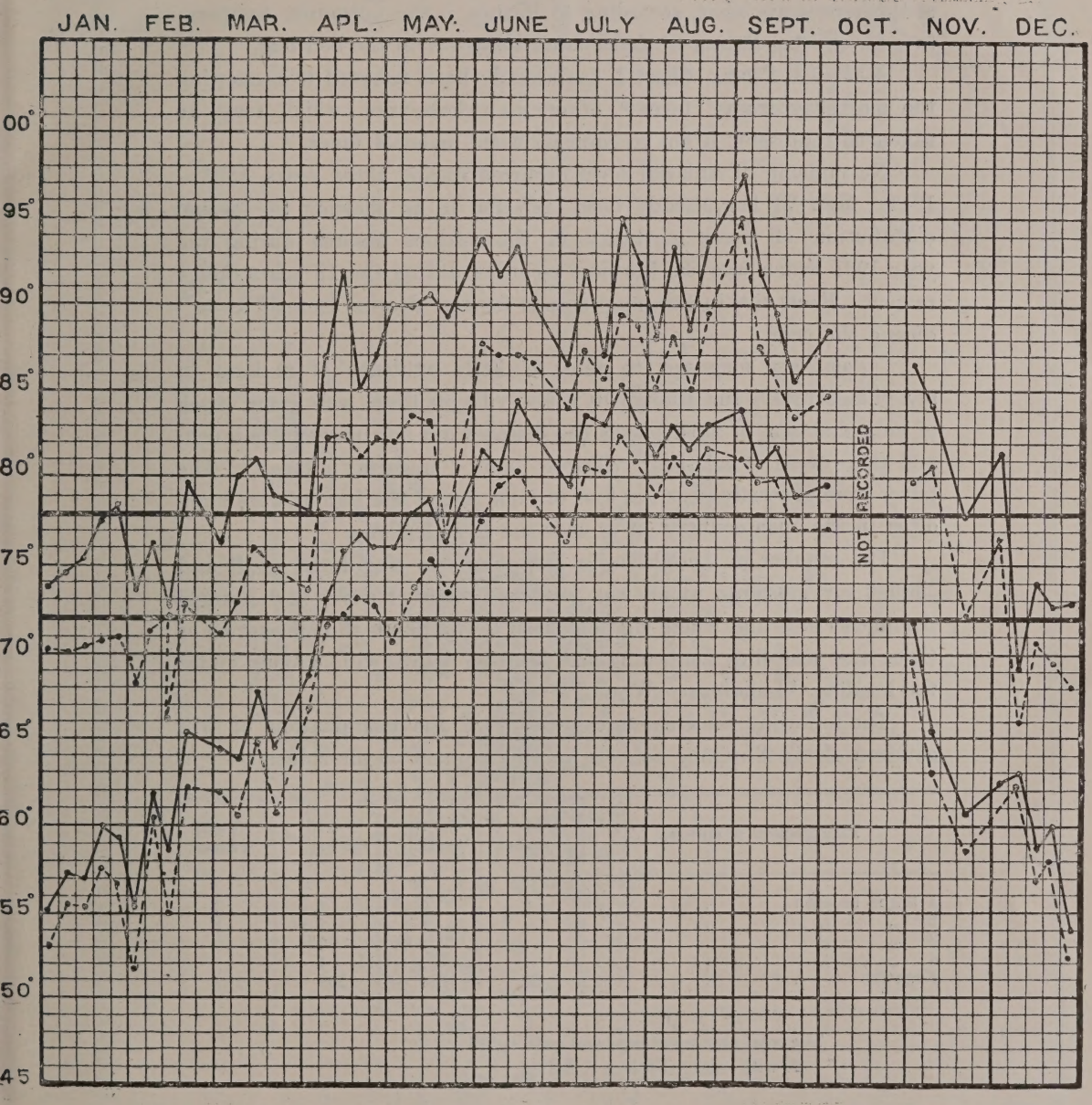


CHART VI.


JAN.
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75°
70°
65°
60°
55°
50°
45
NOT RECORDED

compiled from the continuous thermographic tracings recorded in my office.

The space between 72°—78° F. has been marked out in heavy lines, as this represents what, according to Patton, is the optimum temperature for the flagellation of *Leishmania donovani* in the bed bug.

Malarial Fever.—Chart I has already been referred to as shewing the mortality from "fever" other than kala-azar, which may be taken to represent, if not to coincide with, malarial fever.

No epidemic of malarial fever occurred during these years, 1891—1911. Map VI has already been referred to as shewing the actual distribution of fever mortality in the various *mauzas* during the year 1907 which was the heaviest recorded during the 19 years under review. Each disc in this map stands for ten deaths from "fever" during the year.

I was very surprised to find so little evidence of malaria in my village tours, and though in a few villages there were some cases of both diseases, in most places the diseases did not intermingle much, and there were "kala-azar villages" and "malaria villages." The latter were generally near the foot of hills, whilst the former were more often in the open country on or near the river Kallang.

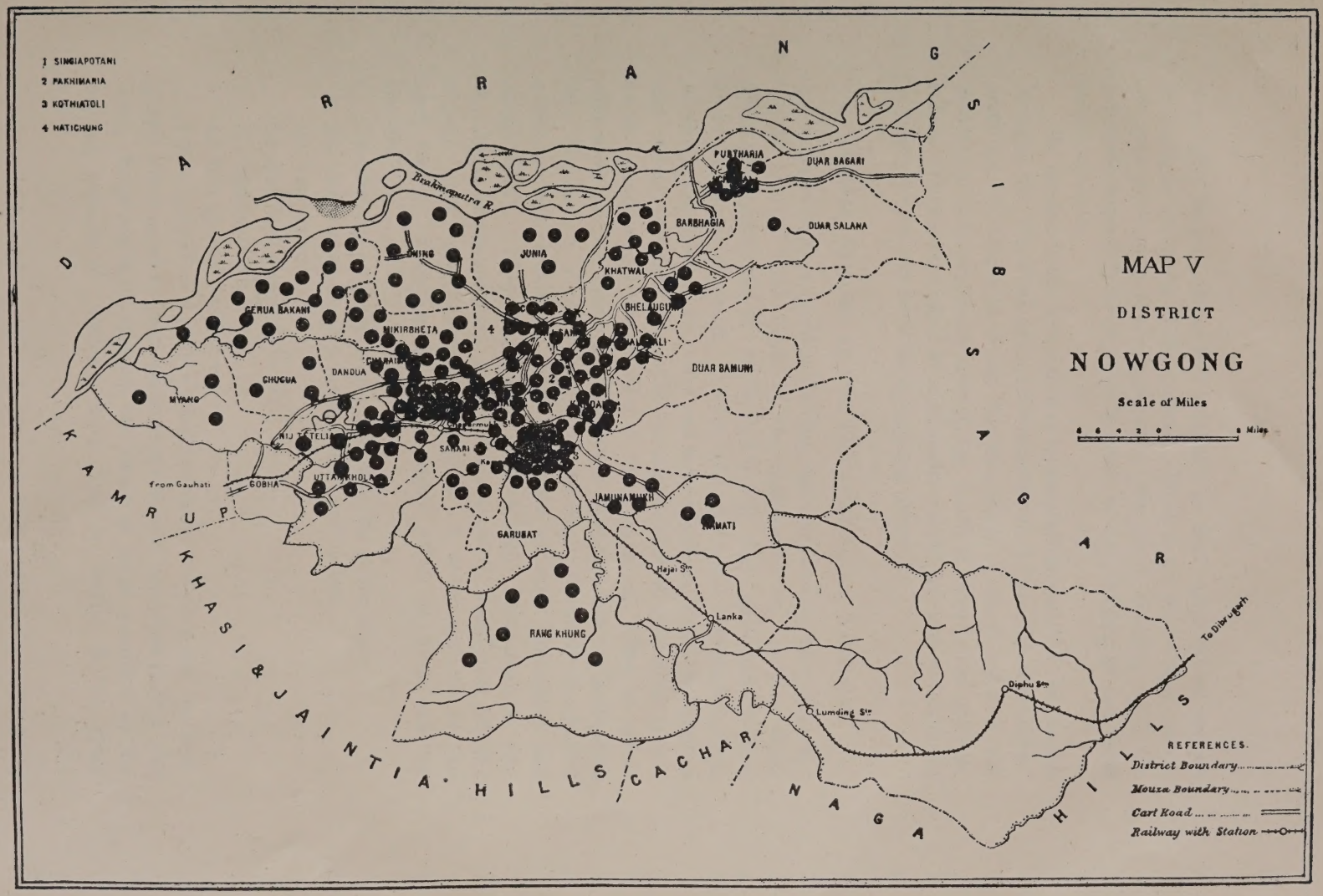
The number of Anopheles in Nowgong was singularly small and though my mosquito room was searched daily, I never saw a single Anopheles in it the whole eight months I was there, though species of Culex and sand-flies were common. Further details will be found under the heading "Laboratory Work."

The following table shews the result of the examination of 573 children of the Nowgong schools for enlargement of the spleen :—

Enlargement of spleen.	Under 5 years.	5—10 years.	11—15 years.	16—20 years.
1 finger breadth	2	5	2	3
2 ,, ,, ...	..	1	2	1
3 ,, ,, ...	..	..	3	..
4 ,, ,, ...	..	..	1	..
Negative	30	194	202	127

The total number of boys and girls = 573
20 with enlarged spleen = 3·5%.

Enlargement of the Thyroid Gland.—Out of the above mentioned 573 children, 18 had unmistakable enlargement of the thyroid gland. A



Shewing the deaths from Kala-azar, during the height of the epidemic in the year 1897.
The total number of deaths this year in this district was 11,552. Each disc represents 50 deaths.

very much larger number had a fullness in that region which seemed to me to be in excess of the normal. There is no doubt that goitres are very common in the villages along the river Kallang and the deformity, especially amongst the women, is very striking to the most casual observer.

My interest in this question was aroused by Captain T. C. McCombie Young, I.M.S., who reminded me of the condition of "parasitic thyroiditis" described by Chagas in South America, which is associated with the presence of *Schizotrypanum cruzi* in the blood of the affected persons. I have examined the blood of some early cases of thyroid enlargement in young people, but have found nothing unusual.

General Remarks on the Epidemiology of Kala-azar.

In my village inspection it has been my custom to collect the "grey beards" and heads of the community, to question them about the disease, and to try to find out if they have any theories as to its mode of spread. I have also got much information from educated Assamese of all castes and have heard many strange opinions and curious theories. It would be unprofitable to give more than a short composite account of what I have gathered. They all agree that, after the big epidemic, the disease subsided and in parts died out, but now it shews signs of reappearing. Formerly it affected adults as well as children, now it affects children principally and rarely adults. They distinguish it accurately from malaria, and the more intelligent can give a "text-book" description of the disease, but they cannot distinguish it from malaria in the early stages. They say it begins most often in June and in October.

The theories most commonly held as to its origin are—

(*a*) Sleeping on the floor instead of a bamboo bed.

(*b*) Bad water.

(*c*) Bad food particularly (*d*).

(*d*) Diseased fish.

(*e*) Bad general sanitation.

I have never heard it attributed to insects and they say the only insects which trouble them are mosquitoes and sand-flies during the rains. Bugs are fairly common but rarely in large numbers, as the bedding consists of nothing more than a closely woven grass mat.

The most commonly held theory is that kala-azar is due to bad water, and the impression is that it is commonest in villages situated on rivers and rare in jungle villages. Our experience bears this out, though the fact that the bulk of the population lives on the river sides has to be remembered.

The articles of diet have been made the subject of enquiry, specially the varieties of fish and the diseases to which they are subject. Some information on the subject will be found under "Laboratory Work."

All are agreed that the starting point of the disease in a community is the coming of an infected person, and even then it is only the persons living with him who are liable to contract the disease. If one married partner contracts the disease, the other is liable to become infected also, and some of the better class cease to cohabit under these conditions. I was informed that sexual intercourse takes place promiscuously amongst children about the age of 11 or 12 onwards, but as the disease is most common before that age this is scarcely likely to be an important factor.

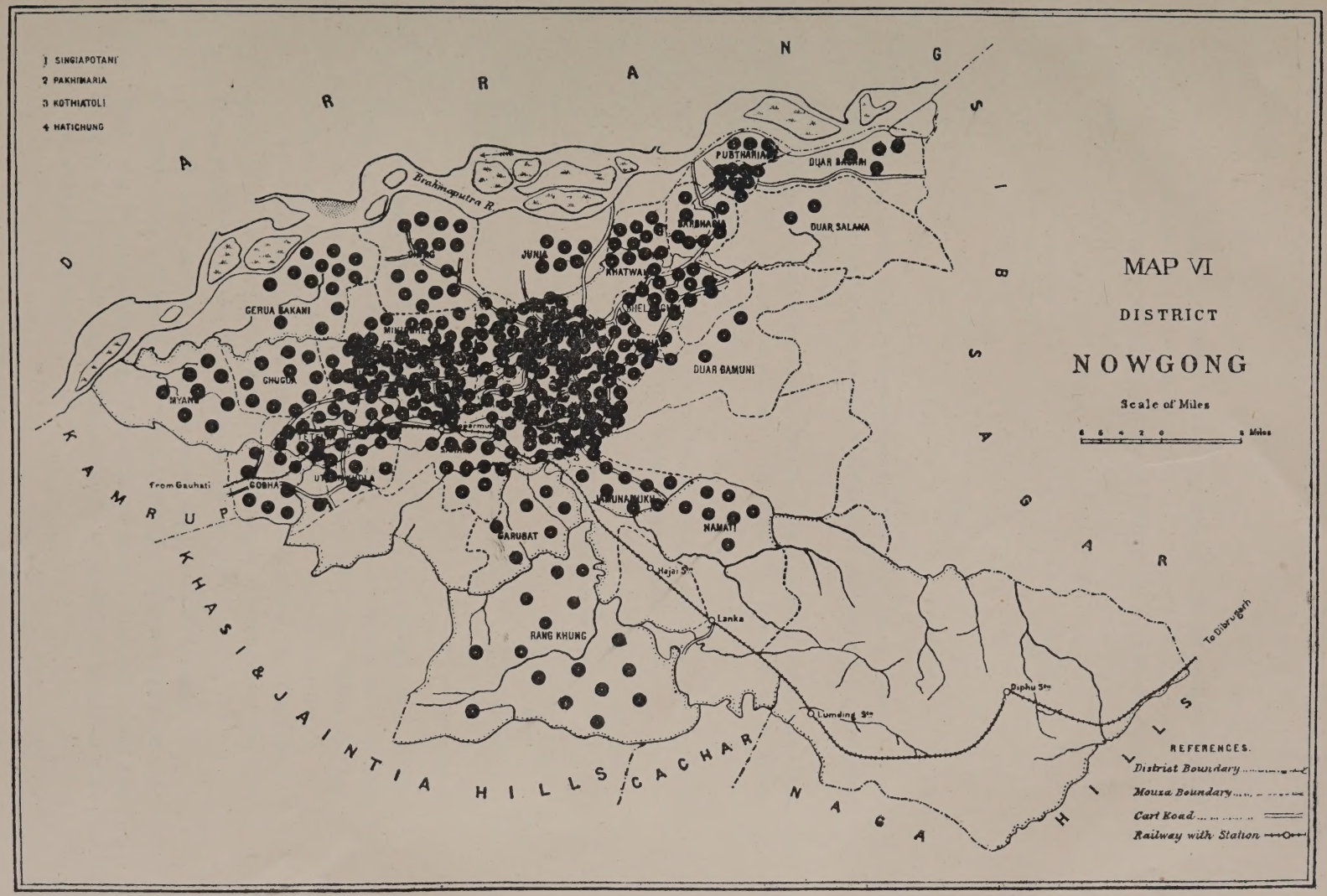
No particular connection can be traced between the disease and the prevalence of healthy or diseased dogs, or with any other animal. Dogs in Assam, as in the East generally, are not household animals, and there is not the same intimacy between them and the children as exists in Europe.

Clinical Observations.

It was our custom to subject every patient who presented himself for treatment to a short but effective clinical examination, and to ascertain, as far as possible, accurate details as to the time and mode of onset and particularly as to the incidence of the disease in the family or village.

I came to the conclusion that much of the history must be taken with great reserve, as from nervousness or from anxiety to please, the details were apt to be vague and often contradictory.

The in-patients numbered in all 32, but as no attempt was made to restrict them they came in and went out as they pleased, and regular clinical observation was difficult. Notes were made on 273 cases which included in and out-patients and some cases seen in the villages, and it was our custom to give a clinical diagnosis to each case at the time



Deaths from "fever" during the year 1907 in which the mortality from this cause was higher than in any other year between 1901—1912.
Each disc represents 10 deaths during the year.

of examination, which was afterwards compared with the results obtained by microscopic examination.

The 273 cases were divided up as follows on clinical examination alone :—

"Typical"	85	
"Probable"	60	205
"Suspicious"	60	
"Doubtful"	68	
TOTAL ..	273	

These terms explain themselves and the next set of figures shews that, when tested by microscope examination, the clinical diagnosis was remarkably accurate.

Some of the cases were subjected to spleen puncture and in all cases peripheral blood was examined. The result in percentage of those examined was as follows :—

Clinical Diagnosis.	LEISHMANIA PRESENT.		Malaria.
	In the Spleen.	In the peripheral blood.	
Typical	87%	20%	0·0%
Probable	93%	20%	2·2%
Suspicious	43%	10%	13·7%
Doubtful	0%	1·4%	18·0%

This analysis enabled one to state in general terms that all the "typical" and "probable" cases were genuine kala-azar, that half the "suspicious" and none of the "doubtful" were kala-azar, but in these two latter classes were grouped all the cases of malaria.

In examining the histories and general symptoms, the "doubtful" cases have been excluded and the percentage calculated on the 205 cases remaining in the first three clinical divisions.

Sex.—Out of 203 persons 123 were males and 80 females.

This does not necessarily mean a greater incidence amongst the male sex, as on approaching a village a few boys would stand fast, and most of the girls and young children would stampede to the huts and evade examination.

Age.—Again, excluding the "doubtful" cases, the ages of 195 persons were recorded. They were grouped as follows :—

Years.					
1—5	..	..	..	..	9
6—10	..	..	..	..	100
11—15	..	..	..	..	49
16—20	..	..	..	..	17
20—30	..	..	..	..	12
Over 31	..	..	..	..	8
					195

That is, 76% were between the ages of 6 and 15 years and 81% in the first three quinquennial periods.

Family Infections.—In all cases some pains were taken to try and trace the origin of the infection, and in the village tours this was supported by personal examination of other members of the family and of neighbours. About half the cases (97 out of 192) gave a fairly clear history of kala-azar, either in the household or among their relatives within five years, but in 95 cases no such history could be obtained. The question of the intensity of family or house infection is a very important one, but our experience shewed that it was very variable; in some villages there was a clear history of a succession of cases in one house, whilst in other villages the cases would be much more scattered, and family infections apparently absent or very rare. The appearance of kala-azar in a village could generally be traced to the coming of an infected person from another village, and if the disease spread, it did so only amongst those brought into intimate personal contact with the new arrival, and the conditions almost always pointed to a personal, rather than to a house, infection. It was not uncommon for a child to contract the disease apparently from playing about in the day time with infected children, even when they did not sleep together at night. This observation, however, is difficult to prove and must be accepted with caution. On the other hand, I have several times been struck with the frequency with which one sees a case of advanced kala-azar, who has for months, or perhaps years, lived in the utmost intimacy with his brothers and sisters, but where the latter shew on examination no sign whatever of disease.

This seems to point either to there being a very low degree of infectivity of chronic cases, or to the fact that the disease can only

spread when given certain unusual conditions. I have endeavoured to construct figures showing the stage of the disease when the infection is most likely to pass, but as such evidence could only rest on the histories given by the surviving relatives, the results are not considered sufficiently accurate to warrant publication. Speaking generally, the terminal stage of the disease seems to be the most infectious, an observation open to more than one interpretation.

Duration of the Disease.—In endeavouring to ascertain the duration of any given case of kala-azar one meets with two great difficulties. One is the ever-present fallacy due to vague ideas of time, and the inaccurate statements of ignorant peasants, and the second is that the onset of the disease is generally so vague that with the best intentions in the world the sufferer is unable to fix it nearer than a few months.

In 250 cases (including all clinical classes) the duration was stated to be as follows : —

Under six months	65
6 months to 1 year	99
1 year „ 1½ years	27
1½ years „ 2 „	33
2 years „ 3 „	14
Over 3 years	12
	250

The chief object in trying to ascertain the exact date of onset was to find out if there was any particular season or month when infectivity was greatest, and, if such a period could have been proved, it might have been found to coincide with a particular set of temperature conditions, or to correspond with the appearance of a particular insect.

The fallacies above referred to were sufficient to prevent any deductions being drawn from the monthly incidence I drew up, and the impression remains that there is no season of the year specially favourable to the spread of kala-azar.

This is also borne out by enquiries of village headmen who are unanimous in saying that malarial fever is commonest during and after the rains, but are vague about the appearance of kala-azar.

Mode of Onset.—From what I have said in the preceding paragraph it is obvious that the mode of onset is generally indefinite. It is indistinguishable at first from malarial fever and is said to be ushered in

with rigors and intermittent or remittent fever. This stage is sometimes well defined, and gives rise to an illness which confines the patient to his hut and prevents him from working. Patients will often state that abdominal symptoms, such as distension and diarrhœa, were noticed, and it seems probable that a proportion of cases exhibit a typhoid-like onset.

After a week or two of irregular fever there is a diminution of symptoms for a few weeks, and then a reappearance of fever, more or less continuous, and the gradual development of the other symptoms. In other cases the intermittent fever passes directly into a low fever of continuous type.

When observed in hospital the double rise of temperature described by Rogers is generally seen. My hospital staff was not large enough to allow me to make any very careful records, but there is generally a rise of temperature in the morning or early afternoon, and a second rise late in the evening or in the early part of the night.

The Alimentary System.—The appetite is capricious; often very small but sometimes very large, and associated with a craving for fish or flesh, a symptom which has been noted by many observers. The tongue is generally clean and moist, and is but little guide to the general state of the patient.

Abdominal symptoms such as diarrhœa or "dysentery" were described in 97 out of 180 cases (53%), and it appears that some bowel trouble is met with at one or other stage of the disease in almost all patients.

The condition described as "dysentery" is common, especially during the later stages of the disease, and is associated with the discharge of mucus and blood, the latter often in considerable quantities. This tendency to hæmorrhage from mucous membranes has not received the emphasis it deserves, especially when the method of escape of the parasite from the body is of such importance.

There was bleeding from the nose or gums in 80 out of 114 cases in which the symptom was enquired about. The bleeding from the gums is generally associated with pyorrhœal gingivitis.

In 134 cases the liver was—

"Palpable" in 27 "Large" ,, 42 "Very large" ,, 19	}	88 = 66%
Not enlarged ,,		46 = 34%

In nine cases the liver was proportionately much larger than the spleen.

The spleen was enlarged in practically all cases, but after excessive diarrhœa or dysentery this organ diminishes in size and may, even, become impalpable.

The regular treatment for expelling ankylostome worms was carried out in 29 in-patients and of these 23 were negative. The six positive cases shewed 10, 12, 13, 7, 5, 13 worms respectively, so that in no case could they be said to complicate the disease.

The rare worms *Gastrodiscus hominis* (Lewis and McConnel, 1876) and *Faciolopsis buski* (Lankester, 1857) were each found on two occasions.

Circulatory System.—Extreme irritability of the heart is a symptom strikingly common in my series and one which has not been referred to much by other writers. The pulse rate on first examination was as follows :—

Under 100 per minute	.. ..	8	= 6·8%
Between 100–119	.. ..	30	= 25·6%
„ 120–129	.. ..	32	= 67·5%
130–139	.. ..	12	
„ 140–149	.. ..	24	
150 and over	.. ..	11	

That is, only 6·8% had a pulse rate of under 100 and as many as 67% had a rate of 120 or over. I look upon a high pulse rate in the absence of fever as a very characteristic sign of kala-azar.

Though high pulse rates were so common, organic disease of the heart was excessively rare ; on the few occasions when murmurs were heard they were always of a hæmic nature and not associated with evidence of valvular mischief.

Examination of the blood was undertaken in the intervals of other work and generally to shew the effect of treatment (salvarsan or vaccines). Attention was in most cases only directed to the leucocyte wave and its variation under different conditions.

Marked anæmia is, in my experience, uncommon in kala-azar and in making a blood film, if the drop is seen to be thin and watery, the case is much more likely to be malaria, post-malarial anæmia, or ankylostomiasis.

Similarly, distortion, poikilocytosis and like changes in the red cells are quite unusual in kala-azar except in terminal cases, whilst normoblasts and megaloblasts have never been observed.

My leucocyte counts have not proved to be so low as those described by some writers, but it would not be accurate to take my figures as comparable with those taken from cases undisturbed by treatment. An average of 24 leucocyte counts gave the following figures :—

Total average leucocyte counts 3562 per cubic millimetre of which (on an average of 33 counts) :—

30·7 % were polymorphonuclears.
60·0 % ,, small mononuclears.
7·5 % ,, large.
1·8 % ,, eosinophile.

The details of these counts will be found in Table I (p. 31).

Peripheral *Leishmania* infections will be referred to under the heading "Laboratory Experiments."

Respiratory System.—A slight bronchitic cough has been noticed in a few cases but pneumonia did not occur in this series. *Leishmania* have been looked for but not found in the sputum.

Cutaneous System.—Pigmentation of the skin is an almost constant sign and in many cases is extreme. The skin in addition is furfuraceous, sometimes almost icthiotic. In a small proportion of advanced cases the skin remained smooth, supple and unpigmented throughout, and in most early cases the skin was unaffected. The hair in advanced cases is rough, harsh and brittle and most of it falls out.

I made careful enquiries into the prevalence of itch, in view of the possibility that the acarine might be concerned in the transmission of the disease. Itch, or what was taken for itch, was found or described as having occurred in 23 out of 120 cases (*i.e.*, 19 %), so this percentage does not support the idea that *Acarus scabei* is a carrier. This insect was diligently sought for in some specially selected cases, but not one was ever found. Owing to scratching and to the chronic nature of the infection, the insect burrows could not be detected and the affected areas were generally crusted and porriginous, which may explain the failure to find the itch mite.

The patients were always carefully examined for sores, ulcers or other lesions which might prove to be cutaneous *Leishmania* infections. Such lesions have been very rare, but when sores or ulcers have been found the examination of smears made from them have always been negative. In a considerable experience of spleen puncture, no nodules or other sign of local infection have ever been noted in the track of the

needle. The attempts made to find *Leishmania* in the leucocytes of blister fluid were negative.

The Urinary System.—No special symptoms were noted under this head. The symptoms of wasting, with a large and voracious appetite, might suggest the possibility of the pancreas being involved and I found that in the early infection of an experimental animal the pancreas was infected with *Leishmania* in a greater degree than the spleen or liver. I examined a few human cases for glycosuria without finding sugar.

Dropsy is rare except in a certain type of case at the end. Oedema of the feet is, however, not uncommon, and transient puffiness of the face has been observed. Ascites has only been seen in three or four cases out of 200, but there is a condition often mistaken for kala-azar in which the liver and spleen are enlarged and the abdomen is full of fluid. Spleen puncture in these cases has always proved negative and their nature is unknown to me.

Prognosis.—I have no figures to show what the present mortality is, but I have seen cases die unexpectedly in whom the prognosis appeared favourable, and others whose weight and general condition have remained constant for a year at a time.

The recovery rate amongst these sporadic cases is certainly higher than that described during epidemic periods. I have seen what others have described, namely, the rapid recovery of advanced cases following some septic complication. One case of this sort struck me particularly—that of a small boy in an advanced state of cachexia who developed a necrotic ulcer burrowing into the left tonsillar area, and who underwent an almost startling change for the better. He improved so much in two weeks that he was unrecognisable. The prevalent organism in the necrotic area was a spirochæte resembling that found in Vincent's angina (unassociated with *Bacillus fusiformis*).

By far the safest guide to progress is the weight—if this is steadily increasing, by however little, the prognosis is favourable, but if regularly decreasing the outlook is bad. No other sign is as reliable as this.

Treatment.—The object of the investigation was primarily to ascertain the mode of spread of kala-azar and the question of treatment was only incidental. I have not found a drug which exerts any influence on the course of the disease, and though recoveries took place now and again I could not credit any line of treatment with this fortunate result. At first our routine treatment was by atoxyl in 2 grain doses

by the mouth daily, and many patients took this treatment, with intervals of rest, for months. Other arsenical preparations were tried, also mercury, quinine, and the usual tonics. Intestinal antiseptics, such as salol and beta-napthol in full doses, produced a good effect in some cases, and eventually this was our routine treatment as it seemed to check the fever and prevent bowel complications. This, together with good food, cod-liver oil and tonics produced the best results.

Vaccines made from streptococci and pneumococci were given a trial with the idea of increasing the polymorphonuclear leucocytes. The blood counts given above shew the result, and in no case was any definite improvement noted. Any form of injection is very unpopular and, if persisted in, will very soon clear the hospital, so that this method of treatment had to be carried out with great circumspection. For this reason neosalvarsan could not be administered by the intramuscular or by the intravenous route. The treatment was offered to several of the better class adults but no one was found brave enough to accept it. Salvarsan was administered by the mouth to three patients, according to the directions given by a writer in a recent number of the *Bulletin de la Societe de Pathologie Exotique*. It gave rise to watery diarrhœa for a few hours but had no effect on the disease nor on the parasites themselves as revealed by spleen puncture. The fact that patients not infrequently recover after such septic complications as cancrum oris suggests the possibility that *Leishmania* are susceptible to the toxines of certain bacteria. I have referred to one case above where the ulceration was associated with spirochætes.

In this connection attention is called to the "Noteworthy case," No. 316, where the *Leishmania* began to dissolve before death and were almost unrecognisable in the spleen three-quarters of an hour after death.

Noteworthy Cases.

Case No. 43.—*Advanced case of kala-azar in a boy unsuccessfully treated with vaccines.*—This boy, *æt.* 7, received in all sixty millions of pneumococci and forty-five million streptococci. Neither the temperature nor the leucocyte count was favourably influenced. *Leishmania* appeared in the peripheral blood and he became worse and was removed from hospital. He developed cancrum oris shortly after returning home and improved, but was still *in statu quo* six months later.

Case No. 50.—*Fully developed case of kala-azar in a young adult treated with vaccines.*—In a period of five weeks 145 million streptococci and ten million pneumococci were injected. No improvement was noticed and the patient six months later was in about the same condition.

Case No. 75.—*Advanced case of kala-azar treated with salvarsan and vaccines.*—The patient received one dose of 0·45 gramme and one of 0·7 gramme of neosalvarsan and on another occasion 20 millions of pneumococci. The course of the disease continued downwards and death followed.

Case No. 84.—*An adult male with acute kala-azar treated with neosalvarsan.*—A man, *æt.* 25, was suffering for 18 months from kala-azar of the hepatic type, that is, the spleen was small and the liver very large. The spleen was punctured but *Leishmania* were not found. The spleen was again punctured a week later and a few *Leishmania* found. 0·45 gramme of neosalvarsan was given by mouth and five days later 0·7 gramme. Beyond producing diarrhœa, these doses had no result. *Leishmania* appeared in the peripheral blood in large numbers (90 in one slide) and persisted till death. He developed purpura of the lower extremities. He remained in hospital for six weeks, was then taken out, and died in his home a few days later.

Case No. 165.—*An early case of kala-azar shewing Leishmania in the peripheral blood.*—Boy, *æt.* 11 years, gave a clear history of four months' fever. There were twelve persons in his house and all except himself were healthy. Stated that his father died six years ago and his step-mother died three months ago of kala-azar. One sister died a month ago after one month's illness. His disease began four months ago with cold and shivering and irregular fever. He has had no abdominal symptoms and has not noticed wasting or darkening of the skin. On examination he seemed healthy except that his spleen was enlarged three finger breadths and the pulse rate was 112 per minute. No skin change, and no sores or itch. The diagnosis of "probably malaria" was made, but two successive examinations of the blood on different days shewed the presence of *Leishmania* in small numbers.

A differential blood count revealed an unusual type of leucocyte in large numbers. These cells were about the size of large mononuclears, but had a single irregular nucleus and a large amount of protoplasm which contained many faint blue granules. They resembled myelocytes

with faint basophilic granules, but were small in size. About 8—10 *Leishmania* were found in three films. The differential blood count was—

Polymorphonuclears	19·0	per cent.
Small mononuclears	29·0	„ „
Large „	19·0	„ „
Myelocyte like cells	32·5	„ „
Eosinophile cells	0·5	„ „

This is the earliest case in which a peripheral infection has been found and the aberrant type of leucocyte present suggests an early stimulation of the bone-marrow.

Case No. 205.—*Case of ankylostomiasis simulating kala-azar.*—A man, aged 25 years, who has been suffering from occasional and irregular fever for five years.

There was no family history of kala-azar, but the general symptoms were not dissimilar to those generally met with in that disease. His skin was pale and smooth, and of yellow colour suggesting profound anæmia. Heart sounds weak with hæmic murmurs; spleen just palpable; liver enlarged three fingers' breadth; one or two small sores were found on feet and ankles; the liver was punctured but no *Leishmania* were found; the peripheral blood was thin and watery, and under the microscope showed changes suggestive of profound secondary anæmia with a large increase of eosinophiles. He absconded before treatment with thymol could be carried out.

Case No. 268.—*An early case of kala-azar who died from rupture of the spleen.*—This woman, age 17, contracted the disease four months after her husband (who had been also under treatment) had died in the Nowgong dispensary. She had been suffering from the disease for ten months but was not an advanced case. She was rather thin, her skin had lost its gloss and her pulse rate was 138. She had no enlargement of the liver but the spleen was enlarged, soft and indefinite. The case was returned as "clinically suspicious" and repeated examination of her blood was negative. I hesitated to puncture her spleen as it was so soft, and in this I was well justified for at the *post-mortem* it was friable and almost diffluent. She fell out of bed one night and was found dead a few hours later.

Post-mortem examination made eight hours after death. No sign of decomposition. The abdominal cavity was full of blood. The spleen was large (2 lbs. 11 oz.) and was deeply indented to form six lobes. Be-

tween the 3rd and 4th lobes there was a large tear extending deeply into the hilum. There was a small accessory spleen. The liver was large (3 lbs. 1½ oz.), but showed no naked eye changes. The abdominal lymph glands and hæmo-lymph glands were soft and enlarged. The rib-marrow was dark and semi-fluid. The alimentary canal was perfectly healthy from end to end, the cæcum contained two ankylostome worms.

On microscopic examination *Leishmania* were found in large numbers in the spleen, liver, bone-marrow, and pancreas. The accessory spleen also contained them. None were found in the hæmo-lymph glands, abdominal lymphatic glands, lung, heart muscle, kidney, ovary or in scrapings from the various parts of the alimentary mucous membrane. The frequency of the parasite in the pancreas and the absence in such organs as the kidney and lung is worthy of note.

Case No. 275.—*Kala-azar of chronic type in an infant.*—A child of three years with a history of illness of a year's duration. There is no information as to how the child contracted the disease; there have been no other cases in the family or amongst the near neighbours.

The child was very thin with a protuberant abdomen and a spleen 2½ fingers' breadth below the costal margin. Liver not enlarged. Skin changes not advanced. Pulse 168.

Spleen puncture revealed an enormous *Leishmania* infection. During the six months the child was under observation bouts of high fever were frequent, but the weight never varied more than 1 lb. or 1½ lb. week by week all the time, and at the end of nearly six months in hospital the patient was not perceptibly better or worse than on admission.

His little sister, age about 10, slept all the time in the same bed, and performed all offices for the child, but she has shown no rise of temperature or other sign of kala-azar.*

Case No. 316.—*A marked case of kala-azar ending in death in which the parasites both in the peripheral blood and in the internal organs were undergoing dissolution at the time of death.*—The case was an ordinary one except for the events which occurred near the time of death.

Spleen puncture on admission 1st May 1913. *Leishmania* of the usual type seen in large numbers.

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* This patient and his sister have been re-examined after a year's interval (25-3-14) and the former is neither better nor worse, and the sister still healthy.

1*st July* 1913.—Parasites seen in peripheral blood.

2*nd July* 1913.—Patient had swelling of right cheek and some inflammation of the inside of the mouth, which was apparently a early condition of cancrum oris.

7*th July* 1913.—This condition of cancrum oris worse but cannot be examined on account of muscular spasm.

8*th July* 1913.—Peripheral blood shewed a considerable number of *Leishmania* but only one here and there was typical ; the majority were losing their outline and the nuclei were swollen up and becoming indefinite. In some leucocytes there were four or five *Leishmania* in a clump scarcely recognisable on account of these changes.

The patient died the same evening and the spleen was removed less than *half an hour* after death. Portions were macerated in sterile saline solution and the emulsion inoculated into the peritoneal cavity of two monkeys, two flying foxes and one puppy dog.* Both the flying foxes and one monkey died next day with signs of peritoneal infection and the second monkey was very sick, but recovered. The splenic emulsion did not show any visible bacterial infection nor did any of the other organs. (Culture materials were not available). When the organs of the patient were examined the same breaking up of the *Leishmania* was evident and in the spleen and bone-marrow only the micro-nuclei of the parasites were found, massed in the endothelial cells, with here and there a *Leishmania* more clearly distinguishable.

The case is significant because it may represent the process of natural cure brought about by cancrum oris. Whether this change was due to the circulation of toxines absorbed from the ulcerated site, or whether it was due to an actual septicæmia, was not proved, but the rapid death of the experimental animals suggested the latter.

As I have already pointed out under "Treatment," a case like this raises the question whether a scientifically graduated and controlled dose of some bacillary products might not bring about more successfully what nature is attempting. Cases have been recorded where *Leishmania,* proved to be present by spleen puncture during life, were not found at the *post-mortem*, and it is probable that had this patient lived a little longer his organs would have been found free of recognisable parasites.

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* The dog developed leishmaniasis as the result of this injection.

Laboratory Work.

Puncture of the spleen was only carried out either to confirm the presence of kala-azar in a village or in suspicious or doubtful cases and in a few instances to test the result of treatment. The cases were divided as follows :—

Leishmania.

	+	—	Total.	% of cases.
Typical	14	2	16	87 %
Probable	14	1	15	93 „
Suspicious	7	9	16	43 „
Doubtful	..	13	13	0·0 „
Total ..	35	25	60	

The technique of the procedure has been simplified so that the operation passes almost unnoticed by the patient. No special precautions were taken and no ill-effect was noticed. The examination of the peripheral blood was carried out in practically all cases and the films, generally two from each patient, were examined for about 15 minutes each. The blood of 217 patients was examined on a total of 433 separate occasions and the percentage of the cases in which *Leishmania* were found was 16·59 or calculating it on the actual number of 433 separate examinations was 11·96.

These cases were arranged as follows :—

Typical	17	20 %
Probable	12	20 „
Suspicious	6	10 „
Doubtful	1	1·4 „

The percentage of cases found to contain *Leishmania* either by visceral puncture or peripheral blood examination was—

Typical	30 out of 85	= 35 %
Probable	24 „ 60	= 40 „
Suspicious	11 „ 60	= 18 „
Doubtful	1 „ 68	= 1·4 „
Total ..	66 out of 273 cases	= 23·8%

Malarial parasites were found in 15 cases out of 217 = 6·8 per cent.

Each of the three types of parasite, benign tertian, malignant tertian and quartan, was present on five occasions.

The percentage of cases in which parasites were found may be summarised as follows :—

Clinical type	Leishmania found in		Malarial parasites found in
	Spleen.	Blood.	Blood.
	%	%	%
Typical	87	20	0·0
Probable	93	20	2·2
Suspicious	43	10	13·7
Doubtful	0·0	1·4	18·0

Excluding the doubtful cases Leishmania were found in the peripheral blood of 35 *out of* 166 *cases, that is in* 21 *per cent.*

Leishmania donovani in the spleen.

The number of parasites seen in films made from spleen punctures is to some extent a matter of chance depending on the amount of splenic pulp taken up and the extent of its dilution with blood.

There is a certain appearance of the parasite which indicates the active progress of the disease, and another type which suggests quiescence. In the first class of cases MULTIPLE *Leishmania* are very common and these represent multiplication forms ; the nuclei are not enclosed in a limiting membrane but are imbedded in pairs in a considerable bulk of faintly basophilic protoplasm and the whole mass is surrounded with a delicate cell membrane. As few as one pair and as many as 15 or 20 pairs of nuclei may be found embedded in a single mass of protoplasm and this cytoplasm is derived, I believe, not from the host cells, but is entirely a product of the parasite.

This condition is quite different from that in which numbers of *Leishmania*, each complete and typical, are found lying in the large nucleated host cells, for the one represents the active growth of the parasite, and the other the natural defence on the part of the host.

In the quiescent cases, generally in large hard spleens of long duration, these multiplication forms are not seen but the parasites are few in number and either scattered singly or in small groups. In these the protoplasm is restricted in amount and the bodies are torpedo or cigar-shaped, flattened or otherwise distorted. When parasites occur in the *peripheral blood* they are invariably contained in leucocytes—though when these are ruptured the *Leishmania* may be found extruded and apparently free. They are about equally common in the large mononuclears and the polymorphonuclears.

The large protoplasmic forms referred to above have been noticed twice in the blood stream and apparently free, and on two occasions

dividing forms were encountered in leucocytes, but generally speaking *Leishmania* in the blood stream are undergoing dissolution.

This opens up a very important question, namely, the significance of *Leishmania* in the peripheral blood, for on it the fabric of the insect transmission theory rests. If the appearance of *Leishmania* in the blood stream signifies, as in the case of the malaria gamete, a preparation for an alternation of generations, then the case for transmission by a biting creature is already half proved. But if, on the other hand, the appearance of *Leishmania* in the blood stream is a fortuitous circumstance, or at most a provision of nature for producing widespread visceral infection, then its significance is not so great. The fact that the parasites are always present in the blood in the interior of phagocytes and that they are generally in various stages of dissolution seems to me to be significant.*

Multiple cell infections are quite common and case No. 84, which shewed the highest peripheral infection (90 in one slide), exemplified this well, for successive multiple infected leucocytes shewed 6, 6, 2, 4, 2, 2, 2, 3, 2 2, 3, 3, 2, 14, 6, 2, *Leishmania* in each cell respectively ; the remainder of the 90 were single.

Parasites are apt to be more frequent in the blood stream towards the termination of a case, but I have not found them more commonly associated with a particular complication than in uncomplicated cases. On the other hand, many advanced cases have not revealed parasites even on repeated examination, whereas occasionally they appear in a shower in quite early and unexpected cases. The earliest case in my series was of 4 months' duration, but the examination of apparently healthy children in an infected house has never revealed an unexpected case.

The blood of some early cases has been examined by dark ground illumination, but no flagellate or other suggestive bodies have been discovered.

The exudate from the gums and smears from cutaneous lesions were examined whenever they were present, but no *Leishmania* were discovered.

The following table gives a summary of the animal experiments carried out (the animals which died of other causes before they could be expected to show infection have been excluded).

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* Since writing the above Mayer and Werner at Hamburg have recorded successful cultivation of *Leishmania* from the peripheral blood. 26-3-14.

Fuller details of these experiments will be found in Table II at the end of this paper.

Summary of Animal Experiments.

	Leishmania.		
Monkeys.	+	—	Total.
By peritoneal route	2	3	5
„ mouth	..	2	2
Still under observation	..	..	7
"Flying foxes."			
By peritoneal route	2	2	4
Other routes	..	2	2
Still under observation	..	..	4
White mice.			
By peritoneal route	3	8	11
White rats.			
By peritoneal route	..	7	7
Still under observation	..	..	4
Other animals.			
Peritoneal route (cat)	..	1	1
Dogs under observation ..	..	..	2
TOTAL ..	7	25	49

The following is a short summary of other laboratory work done :—

In the seven months under review 6,672 fly-papers were placed in kala-azar houses or in the hospital wards. This method of catching insects was used because the prejudices of the people made visits to the interior of their houses unwelcome, and also because it was hoped that insects moving at night would sometimes be caught.

One fly-paper was pinned on the wall in the living room, and a second on the floor near the bed in each house.

The catch of insects was singularly small, and the biting ones were chiefly sand-flies (*Phlebotomus* sp.), species of *Culex*, and a few ticks and fleas.

The insects caught on the fly-papers were dissected, but with the exception of the sand-flies none were found to contain flagellates. An herpetomonad was found to be present in a certain percentage of sand-flies, and will be the subject of a note at a future date.

Mosquitoes.—Anopheles were very uncommon, though some *A. rossii*, *A. fuliginosus* and a few *A. culicifacies* were taken. Sixty-nine were dissected without finding anything.

Some batches were fed on patients who had peripheral *Leishmania*, but no development was observed.

One hundred and twenty-two fleas caught on emaciated dogs from kala-azar villages were dissected ; no flagellates were found.

About 100 ankylostome worms from the fæces of kala-azar patients were dissected and examined, but nothing was found.

Sixty-nine leeches were fed on kala-azar patients whose blood shewed *Leishmania* or had recently shewn them. They were dissected at intervals from one to two months after a single infected feed, but neither flagellates, nor any suspicious bodies were found in any of them.

One leech caught in a tank shewed the presence of a few trypanosomes in its alimentary canal.

Experiments with bugs (*Cimex sp.*).—A considerable amount of attention was paid to the question of bed-bug infection.

In all the village inspections these insects were sought for and when they were found in the bedding of persons with undoubted kala-azar, they were taken to the Laboratory to be dealt with. Bugs of doubtful origin were excluded, and every bug dissected had been taken from the bedding of a positive case. The insects were dissected and the dissection fluid divided into two parts ; one-half was injected into a "Bug Monkey" and the other half examined wet and subsequently dried and stained. A total of 469 bugs caught in houses and 191 young laboratory-bred bugs, who had been fed on patients whose blood shewed *Leishmania*, were examined microscopically and 209 were injected into the peritoneal cavity of monkey, Expt. 49. *Leishmania donovani* was not found in any of the dissected bugs and the monkey is alive and has shewn no sign of infection.* (One monkey into whom material from 93 potentially infected bugs had been injected was carried off by a leopard when the experiment had been going on for six weeks).

The examination of dogs.—93 dogs have been killed in villages and houses where kala-azar is prevalent.

The spleen and bone-marrow of all these dogs have been carefully examined and inoculations done as follows:—

Monkey 39—Bone-marrow of five dogs.
Monkey 45—Bone-marrow of twenty dogs.
Flying-fox 41—Bone-marrow of 5 dogs.
Flying-fox 44—Bone-marrow of 20 dogs.
Dog 42—Bone-marrow of 5 dogs.

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* This animal (Expt. 49) has recently been killed and all its organs examined. No *Leishmania* were found.

These animals are all under observation, but have as yet shewn no sign of disease.* The microscopic examinations were negative in every case.

Examination of fish from the River Kallang.—463 fish comprising 18 different varieties have been dissected and the principal organs examined. In 23 fish small trypanasomes were found. There is no reason to connect these parasites with *Leishmania donovani.* Small fluke-like worms were found to infest the fish called " bami " with considerable regularity. Specimens of these and of the human intestinal worms referred to have been sent to England for identification.

Examination of the fæces.—Bodies resembling *Leishmania* have been found in very small numbers in two out of 40 cases of kala-azar examined, but this observation requires confirmation. Bodies of unknown nature were found in the mucus of a patient suffering from terminal dysentery. They resembled encysted flagellates.

The Government of Assam has shewn its sympathy with the investigation, by providing sums of money to meet the cost of dieting and treatment of kala-azar patients, and also by erecting temporary hospital accommodation in the endemic areas where I elected to work.

In the Nowgong district, my thanks are due to J. W. Dawson, Esq., I.C.S., the Deputy Commissioner, for assistance and advice on many matters, and particularly to Dr. Bancroft, the Civil Surgeon, who kindly placed a ward at my disposal and was unsparing in his efforts to make the enquiry a success.

My assistants were :—
Sub-Assistant Surgeon Jamatram B. Desai (Laboratory) ;
Sub-Assistant Surgeon Diduruddin Ahmed (Clinical) ;
and Mahomed Taslim (Field Entomologist).

My thanks are due to them for steady and loyal work, which was carried on often under conditions of considerable discomfort.

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* These animals have since been proved to be free from infection. (26-3-14.)

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TABLE I.

Blood Counts.

Case number.	Date.	Red cells, per c.m.m.	White cells, per c.m.m.	Date of observation.	Polymorphonuclears, per cent.	Small mononuclears, per cent.	Large mononuclear and transitional, per cent.	Eosinophiles, per cent.	REMARKS.
43 ...	...	...	5312	8-2-13	27	59	12	2	20 millions pneumococci injected, 18-2-13.
Blood + ...	...	...	3437	15-2-13	22	64	9	5	
Spleen + ...	...	...	8437	19-2-13	16	70	13	1	20 millions pneumococci injected, 24-2-13.
...	...	...	2500	25-2-13	15	67	17	3	
...	...	...	2725	4-3-13	34	62	4	0	20 millions pneumococci injected, 28-2-13.
...	...	...	...	8-3-13	12	80	5	3	20 millions streptococci injected, 5-3-13.
									25 millions streptococci injected, 7-3-13.
50 ...	...	...	...	...	...	...	...	...	20 millions streptococci injected, 26-2-13.
Blood — ...	...	...	3343	7-3-13	29	60	9	2	
Spleen + ...	...	...	2406	17-3-13	17	69	13	1	25 millions streptococci injected, 3-3-13.
...	...	...	2500	30-4-13	...	...	...	...	25 millions streptococci injected, 5-3-13.
									25 millions streptococci injected, 7-3-13.
									25 millions streptococci injected, 10-3-13.
									25 millions streptococci.
									10 millions pneumococci injected, 12-3-13.
76 ...	...	...	2500	8-2-13	32	59	7	2	20 millions pneumococci injected, 18-2-13.
Blood + ..	...	...	2187	15-2-13	26	67	5	2	
Spleen + ...	...	...	2500	19-2-13	35·5	50·5	11	3	·45 grain neosalvarsan given by mouth, 3-3-13.
...	...	...	...	8-3-13	29	62	6	3	0·7 grain neosalvarsan given by mouth, 8-3-13.
	...								

TABLE I—*concld.*

Blood Counts—(concld.)

Case number.	Date.	Red cells, per c.m.m.	White cells, per c.m.m.	Date of observation.	Polymorphonuclears, per cent.	Small mononuclears, per cent.	Large mononuclears and transitional, per cent.	Eosinophiles, per cent.	REMARKS.
77 ...	...	...	...	...	...	...	...	...	
Blood + ...	...	...	...	28-2-13	9	79	11	1	
84 ...	...	...	...	...	...	...	...	...	
Blood + ...	...	...	...	8-3-13	15	79	5	1	·45 grains neosalvarsan given by mouth, 3-3-13.
Spleen + ...	...	...	...	9-3-13	36	51	9	3·5	
...	...	...	5312	...	45	64	7	2	·7 grains neosalvarsan given by mouth, 8-3-13.
94 ...	...	...	...	...	..	...	...	...	
Blood + ...	...	...	...	8-3-13	16	72	9	3	0·33 grammes neosalvarsan given by mouth, 8-3-13.
Spleen + ...	...	...	...	...	...	...	...	...	
111 ...	...	...	...	...	...	...	...	...	
Blood — ...	...	4026600	1827	5-4-13	34	57	8	1	Very chronic case.
Spleen + ...	...	2950000	3125	20-3-13	35	60	3	5·1	
160 ...	...	...	...	...	...	...	...	...	
Blood + ...	...	...	...	11-4-13	18	74	6	1	
Spleen + ...	...	...	...	...	...	...	...	...	
	...								
165 ...	...	...	...	...	...	...	...	...	
Blood + ...	...	...	...	14-2-13	19	29	51·5*	0·5	* An unusual form of transitional cell with large amount of cytoplasm containing faint blue granules and a single irregular nucleus.
263 ...	...	...	...	...	...	...	...	...	
Blood — ...	...	...	2812	4-4-13	45	47	6	1·5	
Spleen + ...	...	...	...	...	...	...	...	...	

268 ...	...	...	...	11-4-13	56	38	4	1·5	Fæces contained parasitic fluke.
270 ...	...	...	...	...	...	...	...	...	
Blood − ...	...	2166600	3750	4-4-13	53	41	4	1·0	
Spleen + ...	...	...	...	11-4-13	64	33	2	1·0	
...	...	3660000	2187	4-8-13	46	46	7	1·	
275 ...	...	...	...	11-4-13	25	72	2	1	Infant case.
Blood + ...	...	...	...	19-4-13	16	72	10	1	
Spleen + ...	...	2000000	5473	21-7-13	27	67	4	2	
...	...	...	5967	28-7-13	20	63	16	1	
317 ...	...	...	...	...	...	...	...	...	
Blood − ...	...	3314600	1125	22-7-13	22	74	3	1	
Spleen + ...	...	...	...	...	...	...	...	...	
319 ...	...	3910000 Hg 80%	2800 ...	24-7-13 809	33·5	54·25	8·0	2·25	
Blood − ...	...	...	...	...	...	...	...	...	
Spleen + ...	...	...	...	...	...	...	...	...	
320 ...	...	4416666	8125	23-5-13	46	18	2	34·0	Infection with flukes St. Gastrodiscus.
...	...	...	11,225	5-6-13	51	16	5·5	26·75	
351 ...	...	...	...	...	...	...	...	...	
Blood + ...	...	3516666	5624	22-7-13	42	51	6	1	
Spleen − ...	...	...	...	...	...	...	...	...	
352 ...	...	1983000	3281	30-7-13	49	46	4·6	0·4	Case showing extensive dropsy and anæmia.
Blood + ...	...	...	...	... ...	...	...	...	...	
360 ...	...	2465000	4375	4-8-13	45	48	6·0	1	
Blood + ...	...	...	...	...	...	...	...	...	

TABLE II.

Animal Experiments.

Experimental number.	Source of material.	Route of injection.	Date of experiment.	Animal killed or died on	Result.	REMARKS.
			Monkeys.			
13	Emulsion from human spleen 1½ hours after death.	5 c.c. Intraperitoneal.	21-8-12	1-11-12	+	*Leishmania* found on liver puncture on 24-9-12 and by spleen puncture on 23-10-13 and in the viscera after death.
14	Ditto. ...	Ditto.	21-8-12	31-10-12	–	Liver puncture during life, negative; cultures from viscera, negative.
15	„ ...	10 c.c. by mouth.	21-8-12	29-10-12	–	Cultures negative.
27	Drinking water fouled by the fæces of kala-azar patients daily, for two months.	By mouth.	26-9-12	19-1-13	–	.....
31	Spleen emulsion from monkey 13.	10 c.c. I. P.	1-11-12	19-1-13	–	.....
32	Ditto ...	10 c.c. I. P.	1-11-12	19-1-13	+	*Leishmania* found in bone-marrow, spleen, liver, but not in other organs.
38	Dysenteric discharges from kala-azar patients.	Mouth.	17-3-13 } 31-8-13 }	Alive.	...	No sign of disease.
39	Bone-marrow of five dogs from an infected village.	I. P.	6-2-13	8-7-13	–	Organs are normal.
45	Bone-marrow of 20 dogs from infected area.	I. P.	15-3-13	Alive.	...	Looks healthy.
46	Spleen puncture, human case ...	I. P.	3-4-13	Alive.	...	Looks healthy, liver puncture negative.
47	Ditto.	I. P.	5-4-13	Alive.	...	Liver puncture negative.

49	Intestinal contents from 209 bugs caught in bedding of kala-azar patients.	I. P.	28-4-13 onwards.	Alive.	...	No *Leishmania* in peripheral blood up to date.
51	Spleen emulsion from case 316 ...	5 c.c. I. P.	8-7-13	Alive.	...	.....
53	Ditto. ...	10 c.c. by mouth.	8-7-13	Alive.	...	.....

Flying Foxes (Pteropus edwardsii).

17	Human spleen emulsion ...	3 c.c. I. P.	21-8-12	6-9-12	Negative.	The animal had an abscess at the inoculation site in which *Leishmania* were found, but there was no visceral infection.
18	Ditto. ...	3 c.c. I. P.	21-8-12	13-9-12	+	*Leishmania* found in pancreas, kidney, hæmolymph glands, but not in bone-marrow, spleen or other organs.
29	Spleen emulsion from monkey 13	5 c.c. I. P.	1-11-12	19-1-13	+	*Leishmania* present in spleen liver and bone-marrow, but not in pancreas or kidney.
36	Ditto. ...	10 c.c. by mouth.	1-11-12	19-1-13	–	.....
37	Spleen emulsion of monkey 13 ...	Rubbed into scarified skin.	1-11-12	19-1-13	–	No cutaneous lesion.
41	Bone-marrow of 5 dogs ...	I. P.	3-3-13	Alive.	...	Peripheral blood negative.
44	Bone-marrow of 20 dogs ...	I. P.	15-3-13	Alive.	...	Ditto.
48	Spleen juice, human case 263 ...	I. P.	8-4-13	28-8-13	–	Hæmamœbæ in large numbers; no *Leishmania*.
48 (α)	Ditto, ditto case No. 160.	I. P.	10-6-13	Alive.	...	Suckling of Bat 48.
56	Spleen emulsion from case 316 ...	By mouth.	8-7-13	Alive.	...	.....

TABLE II—(*concld.*)

Animal Experiments—(concld.)

Experimental number.	Source of material.	Route of injection.	Date of experiment.	Animal killed or died on	Result.	REMARKS.
			White mice.			
1	Spleen juice from human cases ...	I. P.	31-7-12	15-8-12	–	.....
2	Ditto.	I. P.	1-8-12	20-8-12	–	.....
4	Ditto.	I. P.	8-8-12	18-8-12	–	.....
6	Ditto.	I. P.	19-8-12	15-9-12	+	A few *Leishmania* found in bone-marrow but none in other organs.
9	Ditto.	I. P.	21-8-12	27-9-12	–	.....
10	Ditto.	I. P.	21-8-12	28-8-12	+	A few *Leishmania* in spleen ; but none in other organs (infected in a week).
11	Ditto.	I. P.	21-8-12	28-9-12	–	.....
12	Spleen emulsion from human case	I. P.	21-8-12	15-9-12	–	.....
26	Spleen puncture juice ...	I. P.	11-9-12	18-9-12	–	.....
33	Spleen emulsion from monkey 13	1.5c.c. I. P.	1-11-12	19-1-13	+	Few *Leishmania* in bone-marrow ; absent in other organs.
34	Ditto. ...	1.5c.c. I. P.	1-11-12	19-1-13	–	.....
			White rats.			
19	Spleen emulsion from human case P. M.	3c.c. I. P.	21-8-12	25-9-12	–	Microscopic examination and culture negative (Row's medium).
20	Ditto. ...	Do.	Do.	26-9-12	–	Ditto.
21	Ditto. ...	Do.	Do.	10-9-12	–	Ditto.

22	Ditto. ...	Do.	Do.	24-9-12	–	Ditto.
23	Ditto. ...	Do.	Do.	25-9-12	–	Ditto.
24	Ditto. ...	Do.	Do.	24-9-12	–	Ditto.
25	Spleen emulsion of mouse 10 ...	I. P.	28-8-12	26-10-12	–	Ditto.
58 59	Fresh spleen tissue—human case	By mouth.	8-7-13	Alive.	...	.....
60	Human spleen juice ...	I. P.	31-7-13	Alive.	...	.....
61	Ditto. ...	I. P.	31-7-13	Alive.	...	.....
62	Intestinal contents of 6 leeches fed on kala-azar patients on 17-7-13.	I. P.	7-8-13	Alive.	...	.....
Cat 35.	Spleen emulsion from monkey 13	I. P. 5 c.c.	1-11-12	19-1-13	...	.....
Dog 42.	Bone-marrow of 5 dogs ...	I. P.	3-3-13	...	...	Liver puncture negative.
	6 c.c. of spleen emulsion from K.-A. case 316.	I. P.	8-7-13	Alive.	...	.....
Dog 63.	Dysenteric mucus from human kala-azar case.	By mouth.	10-8-13	Alive.	...	.....